Statement upon claim rejection concerning P107901US

Natural occurring phospholipids, as PC, PE, PG, PI, PA and PS, are molecules comprising of polar head groups (hydrophilic) and non polar membrane anchor chains (lipophilic). Such structures are routinely named as amphipatic or amphiphilic, due to their ability to organize themselves in watery (polar) environment. The polar head group always consists of a negative charged phosphate (→ phospholipid) and variable additional neutral groups (as in PG, PI and PA), positive charged groups (as in PC and PE) or zwitterionic groups (as in PS).

PC (Lecithins from Egg or Soya and synthetic Phosphatidylcholines) as well as PE (Phosphatidylethanolamines) are phospholipids comprising of the said negative charged (anionic) phosphate group and a positive charged (cationic) ammonium group (choline in case of PC) or amine group (ethanol amine in case of PE). Structures, comprising of such combinations of negative and positive groups are routinely named as zwitterionic molecules. Within a range of environmental conditions, the above mentioned charge groups are fixed and not sensitive to changes. The sensitivity of the charge, however, is the most important property of the so called amphoteric molecules. At least one of the two charged groups must be sensitive to the relevant environmental conditions. The relevant condition is stated as the pH, which is directly related to the concentration of protons.

In figure 1-3 (extracted from the web page of one of the most important and famous lipid supplier company in the world = Avanti Polar Lipids), the dependence of the overall charge of the relevant phospholipids from the pH of the environment is shown. The physiological pH is about 7.4 (in blood) and the pH range for pharmaceutical use of phospholipids is between 3 and 9. Outside this pH range, phospholipids tend to become unstable and cleavage of ester bonds occurs. Taken this, PC and PE are not sensitive towards the useable pH range, thus they do not provide the most important property for constructing amphoteric liposomes (although they are sometimes very helpful as membrane building lipids). Therefore, PC and PE are routinely described as zwitterionic, but neutral lipids.

pH sensitivity is related to the existence of charge switchable groups. These groups may be divided into cationic (or alkaline) groups and anionic (or acidic) groups, often independent whether they are linked to the same or different membrane anchor.

pH sensitive anionic groups are carboxylic groups or distal (end standing) phosphate groups, but not endophosphate groups as they are present in PC and PE.
pH sensitive cationic groups are secondary and temary amino groups or imidazole groups, but not quaternary ammonium groups as being part of PC. The pH sensitivity of primary amino groups as being part of PE is above pH 9 and therefore outside the pharmaceutical range.

Taken the said into account, we want to present a table, listing the used ionic groups of the different patents, which are currently positioned as critical to our invention.

Summarizing the individual columns, one may extract that none of the critical patents are bearing the inventional amphoteric property. To receive an amphoteric character, the respective lipid or liposome must be able to respond to the pH environment in a way that at one pH the structure has an overall positive charge and at another pH an

overall negative charge. As a consequence, there is a pH in between the above said where the structure has a net neutral charge. That pH is routinely described as the isoelectric point. Having only anionic or cationic lipids as pH sensitive components in the liposome membrane, never results in structures which are able to switch from positive to negative net surface charge in reaction to environmental pH changes. They only can shift from positive or negative towards neutral surface charges.

Positive and negative charge carrier must not be inherently different. The positive and negative charge group can be attached to the same membrane anchor. That, for example happens if you use the natural aminoacid histidine as the polar head group, attached to a lipophilic membrane anchor via the alpha-amino group. Thereafter you will have an amphipatic molecule with one positive charge group (here imidazole group) and one negative charge group (here carboxy group). Such amphoteric liposomes, consisting only of amphoteric charge carriers are claimed in Claim 3. What we claimed in Claim 1, is an amphoteric liposome, comprising of charge carrying lipid components, where the positive charge group is attached to one membrane anchoring molecule and the negative charge group to another membrane anchoring molecule, thus being two different molecules. These different lipid molecules are essential parts of the amphoteric liposome. They are claimed to be simultaneously (Claim1 – "and"), rather than exclusively ("or"), part of the whole liposome.
We further claimed in Claim 5 a combination of amphoteric charge carriers and anionic and/or cationic charge carriers. The isoelectric point of the amphoteric charge carriers will affect the respective value of the whole liposome, but these charge carriers are relevant players in the "isoelectric story".

The isoelectric point (being in between 4 and 8, or even 5 and 7) is a property of the claimed liposomal structure, which itself consists at least out of the individual charge carriers according to Claim 1/2, 3/4 or 5/6. Further neutral lipids (according to Claim 7) may be, but must not be necessarily part of the amphoteric liposome. As outlined above, these neutral lipids, even if they are zwitterionic, do not affect the isoelectric point of the whole amphoteric liposome.

The claimed sizes of the liposomes (recited in claim 8 after the terms, preferably and particularly) are indeed the limitations, regarding their use as pharmaceuticals for effective intracellular delivering of active ingredients after systemic application. Due to the claimed method for charging liposomes with active ingredients (Claim 12), the liposomes are of unilamellar structure.

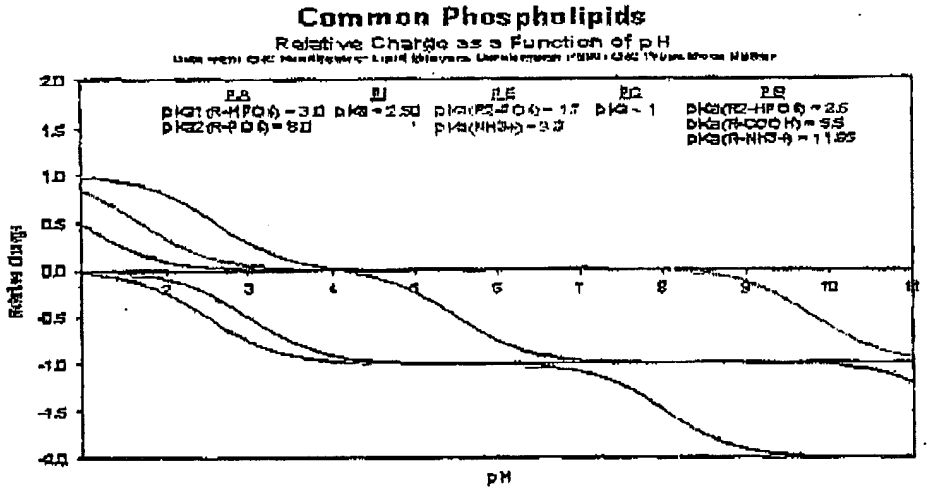

Figure 1

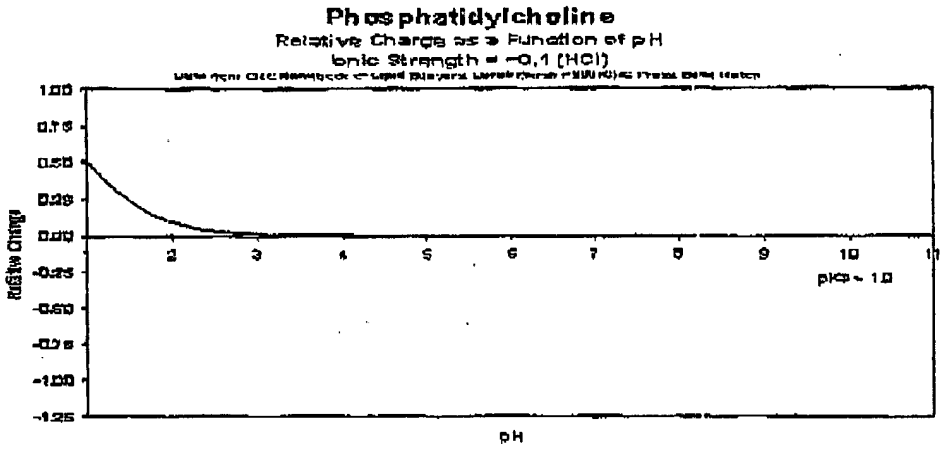

Figure 2

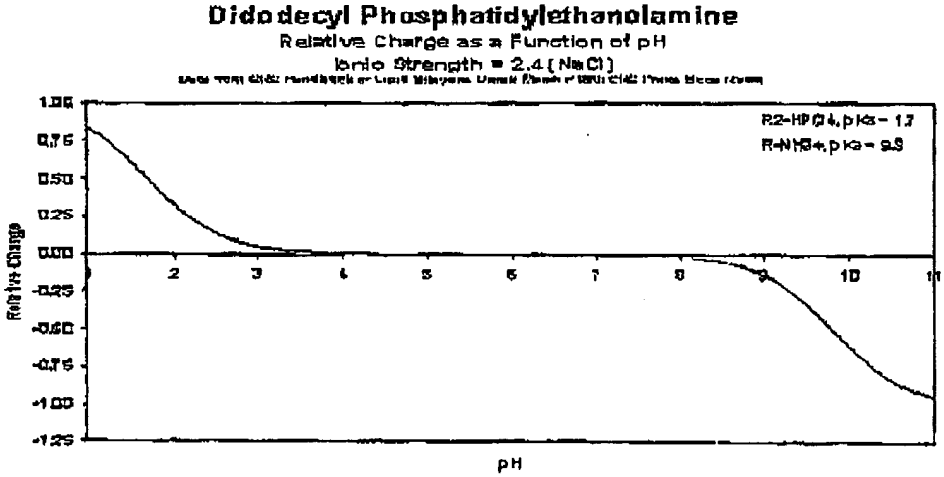

Figure 3

Definition of amphoteric structures

The amphoteric situation is defined as a structure which behaves simultaneously as an acid and a base, depending of the pH of the environment. Therefore structures which can be described as amphoteric must consist of acidic and alkaline groups or molecules.
Under practicable conditions, the acidic and alkaline properties must be achievable at lipid compatible conditions, which means between a pH of 3 and 9, otherwise existing ester bonds may be cleaved.
Being a zwitterionic substance or structure is necessary but not sufficient to fulfil the above claim. Thus, neither PC nor PE can be considered as amphoteric lipids. It seems to me, that somewhere in the literature, the items "amphoteric" and "amphipatic" are used in the wrong way. The item "amphipatic" correctly describes the simultaneous polar and nonploar property of those lipids and many other lipids and tensids, but has nothing to do with the charge property.
Indeed, if PC and/or PE would really be amphoteric lipids, than one may be able to construct amphoteric liposomes out of these lipids. **BUT**; PC and PE can in no way be described as amphoteric lipids, especially in the practical pH range between 3 and 9. In between this pH range, PC and PE are zwitterionic lipids, but with an overall neutral charge and absolutely not sensitive to pH modifications in that range (see Figure 1-3 in my first letter).
Therefore, one will not be able to construct amphoteric liposomes, with the said properties, out of PC and/or PE.

You may check in the respective US literature for a definition of the item "amphoteric" or go back to the Greek origin of the item. Unfortunately, I was not able to find such a definition in English language, only in German. Therein "amphoteric" is defined as "a substance, which behaves as an acid, as well as a base, according to the environment" (free translation).

Table for comparison of the invention with the cited prior art (C=claim number, Ex=example number)

Invention (claim 1)	Millet-Gerlin	Dees	Delneu	Kripke	Yarosh
Amphoteric liposomes, wherein the liposomes	No (see further explanation in text)	No (see further explanation in text)	No (see further explanation in text)	No (see further explanation in text)	No (see further explanation in text)
comprise at least one positive charge carrier and	Stearylamine (C5) PS at pH<2 (C9) OR	Stearylamine (C2)	Alkylated quaternized polysaccharide (C1)	none	Stearylamine (E3) OR
at least one negative charge carrier which is different from the positive charge carrier,	Dicetyl-P (C6) PS at pH>6 (C9)	none	none	Oleic Acid (C4) Chems (Ex1)	Chems (C11) Oleic Acid (C13) DCP, PG (Ex4)
the liposome having an isoelectric point of between 4 and 8	Either > 9 Or < 2	> 9	none	< 5	Either > 9 Or < 5
further comprising neutral amphiphilic lipid (claim 7)	Chol (C1) PC (C7)	PC (C2) Chol (C4)	PC (C8)	PC, PE, Chol (C4)	PC, PE (C10) Chol (Ex4))